

## SEXUAL STERILITY is Essential for Both Male and Female Gametogenesis in Tomato

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journal or publication title	Plant and cell physiology
volume	58
number	1
page range	22-34
year	2017-01
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URL	<a href="http://hdl.handle.net/2241/00145944">http://hdl.handle.net/2241/00145944</a>

doi: 10.1093/pcp/pcw214

Supplementary Table S1. The mutations in the *Sleses* mutant

Chr	Position	Reference (Micro-Tom)	<i>sleses</i> mutant	Mutation type	Indel length	DP4 (V,W,X,Y)	Locus	ITAG number	Annotation	Mutation	Effect of mutation
62,468,747	T		TC	IN		1 0,0,3,4	IGR				
62,475,310	GT		G	DEL		-1 0,0,17,25	Intragenic region Solyc07g06257(Ubiquitin-conjugating enzyme E2 N			intron	
62,482,892	C		CAAAATGACT	IN		9 0,0,11,2	IGR				
62,485,918	AG		AGG	IN		1 0,0,1,1	IGR				
62,501,963	TC		T	DEL		-1 0,0,1,1	IGR				
62,501,964	CTTTTTTTTT		CTTTTTTTTT	IN		1 0,0,5,4	IGR				
62,525,634	GAAAAAAAAAAAA		GAAAAAAAAAAAA	DEL		-1 0,0,7,13	IGR				
62,613,928	AT		ATCT	IN		2 0,0,1,2	IGR				
62,614,410	TAAAAAAAAAAAAA		TAAAAAAAAAAAAA	IN		2 0,0,0,10	IGR				
62,618,272	CAAAAAAAAAAAAA		CAAAAAAAAAAAAA	IN		1 0,0,9,8	IGR				
62,621,533	CTTTTTTTTTTT		CTTTTTTTTTTT	IN		2 0,0,9,19	IGR				
62,664,178	CTTTTTTTTT		CTTTTTTTTT	IN		1 0,0,5,12	IGR				
63,002,908	CAAAAAAAAAAAAA		CAAAAAAAAAAAAA	DEL		-1 0,0,23,15	IGR				
63,008,548	T		C	SNP		13,21,168,247	IGR				
63,020,867	GAAAA		GAAA	DEL		-1 0,0,0,4	Intragenic region Solyc07g06329( Unknown Protein			intron	
63,033,736	G		GATGT	IN		4 1,0,8,15	Intragenic region Solyc07g06332( LanC-like protein 2			intron	
63,035,794	C		G	SNP		0,0,2,1	IGR				
63,037,848	GGAG		GAGAG	IN		9 0,0,2,1	IGR				
63,045,924	AGTG		AGAATTAACGGTG	IN		9 0,0,11,14	IGR				
63,053,924	A		AAAAC	IN		4 0,0,4,2	IGR				
63,058,609	TT		TTCAT	IN		3 0,0,3,1	IGR				
63,070,286	CA		CAGA	IN		2 0,0,3,0	IGR				
63,070,690	T		TAAA	IN		3 0,0,1,2	IGR				
63,080,600	G		A	SNP		0,0,1,2	IGR				
63,088,782	CAAGAAGCGAAAAG		CAAG	DEL		-10 0,0,2,0	Intragenic region Solyc07g06339( Beta-glucosidase			intron	
63,091,155	CAAAAAAAAAA		CAAAAAAAAAAAAAA	IN		7 0,0,2,1	IGR				
63,091,163	AGG		A	DEL		-2 0,0,1,1	IGR				
63,091,165	GAAAAAAAAA		GAAAAAAAAA	IN		2 0,0,3,4	IGR				
63,091,769	C		CT	IN		1 0,0,1,2	IGR				
63,091,881	C		T	SNP		0,0,2,0	IGR				
63,092,103	A		C	SNP		0,0,4,0	IGR				
63,102,932	AGG		AGGG	IN		1 0,0,2,2	IGR				
63,104,712	TG		TTAATTCAG	IN		7 0,0,17,13	IGR				
63,105,333	CAAAAAAAAAA		CAAAAAAAAAAAAAA	IN		4 0,0,2,3	IGR				
63,105,358	AG		A	DEL		-1 0,0,1,1	IGR				
63,108,880	AAA		AAAACAA	IN		4 0,0,14,14	IGR				
63,113,058	GAAAAAAAAA		GAAAAAAAAA	IN		1 0,0,8,11	IGR				
63,126,005	TAAAAA		TTAAAAA	IN		2 0,0,22,18	Intragenic region Solyc07g06342( NAC domain transcription factor			intron	
63,129,991	TAAAAA		TAAAAA	DEL		-1 0,0,6,7	IGR				
63,137,609	GTTT		GTT	DEL		-1 0,0,0,3	IGR				
63,182,669	A		ATATTTTC	IN		7 0,0,5,8	IGR				
63,183,264	TAAAAA		TAAAAA	IN		8 0,0,2,5	IGR				
63,196,959	AAAA		AAAAGAAA	IN		4 0,0,6,4	IGR				
63,197,263	T		TCAAAAAACA	IN		9 0,0,3,0	IGR				
63,197,574	AATA		AA	DEL		-2 0,0,0,2	IGR				
63,198,726	AC		A	DEL		-1 0,0,14,20	IGR				
63,207,057	AATTT		AATTTAAAGAGCATATGATT	IN		25 0,0,3,1	IGR				
63,211,620	GAAA		AAGGATATTT	IN		1 0,0,1,2	Intragenic region Solyc07g06352( Transmembrane protein 34			intron	
63,213,768	TTGT		T	DEL		-2 0,0,2,2	Intragenic region Solyc07g06353( Unknown Protein			intron	
63,217,639	T		TG	IN		1 0,0,3,1	Intragenic region Solyc07g06353( Unknown Protein			3'UTR	
63,218,656	CT		CTT	IN		1 0,0,0,4	IGR				
63,224,093	CTTT		C	DEL		-3 0,2,12,6	Intragenic region Solyc07g06355( Arf-GAP with GTPase, ANK repeat and PH domain-containing protein 1			intron	
63,224,569	CTATGGAATA		C	DEL		-9 0,0,7,1	Intragenic region Solyc07g06355( Arf-GAP with GTPase, ANK repeat and PH domain-containing protein 1			intron	
63,230,602	ATTTTTT		ATTTTTTTTT	IN		3 0,0,0,4	IGR				
63,233,770	C		T	SNP		0,0,3,1	Intragenic region Solyc07g06356( Cotton fiber expressed protein 1			3'UTR	
63,255,279	T		TA	IN		1 0,0,0,4	Intragenic region Solyc07g06359( Myosin-like protein			intron	
63,263,762	C		CG	IN		1 0,0,1,1	Intragenic region Solyc07g06360( Chlorophyll a-b binding protein 13, chloroplast			intron	
63,264,622	A		T	SNP		0,0,2,2	IGR				
63,271,176	TCCC		TCC	DEL		-1 0,0,1,1	IGR				
63,276,145	TAAAAA		TAAAAA	DEL		-1 0,0,5,3	IGR				
63,276,316	TTTTT		TTTTTGT	IN		6 0,0,13,6	IGR				
63,279,307	GT		GTAT	IN		2 0,0,0,4	IGR				
63,285,846	T		TA	IN		1 0,0,1,1	Intragenic region Solyc07g06365( Ubiquitin carboxyl-terminal hydrolase			intron	
63,286,441	AC		ACACTC	IN		4 0,0,3,0	Intragenic region Solyc07g06365( Ubiquitin carboxyl-terminal hydrolase			intron	
63,286,442	CTC		CACTCTCAGAAAGCCAAGGC	IN		20 0,0,11,11	Intragenic region Solyc07g06365( Ubiquitin carboxyl-terminal hydrolase			intron	
63,297,255	CAAAAA		GTC	IN		1 0,0,9,3	IGR				
63,299,377	T		C	SNP		0,0,0,6	IGR				
63,301,438	G		GTATTT	IN		5 0,0,2,1	IGR				
63,302,667	ATT		A	DEL		-2 0,0,3,0	IGR				
63,303,142	GAAGAAGCAACCACAA		GAA	DEL		-13 0,0,12,13	Intragenic region Solyc07g06367( Unknown Protein			exon	Frame shift
63,304,222	T		A	SNP		0,0,1,3	Intragenic region Solyc07g06367( Unknown Protein			exon	Missense
63,309,151	T		TCCTC	IN		4 0,0,25,14	Intragenic region Solyc07g06368( CHP-rich zinc finger protein-like			intron	
63,311,339	ATTTCACT		A	DEL		-7 0,0,4,0	IGR				
63,313,247	CTTTTTTTTT		CTTTTTTTTT	IN		1 0,0,4,4	IGR				
63,437,474	ATTTTTTTTTTT		ATTTTTTTTTTTTT	IN		2 0,0,5,12	IGR				
63,444,260	CAAAAAAAAAA		CAAAAAAAAAA	IN		1 0,0,4,18	IGR				
63,473,474	CTTTTTTTTTTT		CTTTTTTTTTTT	IN		1 0,0,12,8	IGR				
63,508,655	TAAAAAAAAAAAAA		TAAAAAAAAAAAAA	IN		3 0,0,2,7	IGR				
63,862,271	TAAAAAAAAAAAAA		TAAAAAAAAAAAAA	IN		2 0,0,10,9	Intragenic region Solyc07g06455( Genomic DNA chromosome 5 P1 clone MBG			intron	
63,948,376	CATATATATATATATATATA		CATATATATATATATATATA	IN		2 0,0,3,2	IGR				
63,977,710	CTTTTTTTTTTT		CTTTTTTTTTTT	IN		1 0,2,13,17	Intragenic region Solyc07g06472( GDSL esterase/lipase At5g55050			intron	
64,027,233	A		AAG	IN		2 0,0,4,1	IGR				
64,036,652	ACT		AAACCT	IN		3 0,0,2,0	IGR				
64,036,653	CTA		CA	DEL		-1 0,0,3,1	IGR				
64,069,862	CAAAAAAAAAAAAA		CAAAAAAAAAAAAA	IN		1 0,0,8,6	IGR				
64,079,800	ACCCCCCCCCCCCC		ACCCCCCCCCCCCC	IN		6 0,0,1,2	IGR				
64,082,904	TAAAAA		TAAAAA	DEL		-1 0,0,4,1	Intragenic region Solyc07g06488( Small ubiquitin-related modifier			intron	
64,094,485	TA		TCAAAA	IN		4 0,0,3,5	IGR				
64,136,178	ATTTTTTTTTTT		ATTTTTTTTTTT	IN		1 0,1,5,9	IGR				
64,265,168	TTATATATATATATATATAT		TTATATATATATATATATAT	IN		41 0,0,3,0	IGR				
64,298,439	ATATATATATATATATATAT		ATATATATATATATATAT	IN		1 0,0,13,9	IGR				
64,421,532	GATATATATATATATATATA		GATATATATATATATATATA	IN		38 0,0,5,3	IGR				
64,593,337	CA		CAA	IN		1 0,0,4,2	Intragenic region Solyc07g06570( SEC14-like protein 1			exon	Frame shift
64,611,647	CTTTTTTTTTTT		CTTTTTTTTTTT	IN		1 0,2,15,15	IGR				

Chr Position; physical position of mutation on the tomato reference genome (SL2.40). Reference (Micro-Tom); Sequence of WT tomato Micro-Tom genome (SL2.40). DP4; Four numbers correspond to number of forward ref alleles, reverse ref, forward non-ref, and reverse non-ref alleles, used in variant calling. Locus, mapped position for *s/sep* mutation. Mutation position; Exon indicates that homozygous indel or SNPs found in *s/sep* mutant are located within the exon region, while intron indicates that the indel or SNPs are located within the intron region. IGR, intergenic region. ITAG name, ITAG gene number defined in SL2.40. Effect of mutation; Missense mutation causes non-synonymous mutation. Frame shift indicates the gap of amino acid transration.

Supplementary Table S2. List of primers

Primer name	Purpose	Forward Primer Sequence	Reverse Primer Sequence	Restriction Enzyme
<i>SISES</i> marker-1	DNA marker ( <i>SES</i> mutation)	CGAGTAAAAATGGACAGAAGAAGAA	TGATGAAACAGGAGTTAATTTAGGG	-
<i>SISES</i> marker-2	DNA marker ( <i>SES</i> mutation)	ACACCATAGCTAGCCAGACCATGT	ACAATGAGCCACCAGTTGAAGCTC	<i>Bsl</i> I
<i>NPT</i> II marker	DNA marker ( <i>NPT</i> II)	ATGATTGAACAAGATGGATTGCAC	TCAGAAGAACTCGTCAAGAAGGCG	-
Whole <i>SISES</i> gene	In-Fusion	GATTACGCCAAGCTTCAATTAGCGATGGACGAAGTICTAGGACGCGTCGACCTCACTTCTAGACTGCCATAAAGC		-

Supplementary Table S3. Homology with SISES

Protein		Identity	Similarity	Gaps
SPL/NZZ	AT4G27330	26.4%	39.2%	33.7%
TIE1	AT4G28840	12.6%	21.3%	60.0%
TIE2	AT2G20080	12.9%	18.4%	67.9%
TIE3	AT1G29010	21.1%	31.8%	36.3%
TIE4	AT2G34010	17.1%	28.3%	38.0%

Each Homology was calculated with The European Bioinformatics Institute.

SPL/NZZ, SPOROCTELESS/NOZZLE

TIE, TCP INTERACTOR CONTAINING EAR MOTIF PROTEIN

Supplementary Table S4. *t*-butyl alcohol series

	<i>t</i> -butyl alcohol	Ethanol	DW
1	10 mL	40 mL	50 mL
2	20 mL	50 mL	30 mL
3	35 mL	50 mL	15 mL
4	55 mL	45 mL	0 mL
5	75 mL	25 mL	0 mL
6	100 mL	0 mL	0 mL

Each solution are composed with *t*-butyl alcohol, ethanol and distilled water (DW).

Supplementary Table S5. List of primers for qRT-PCR

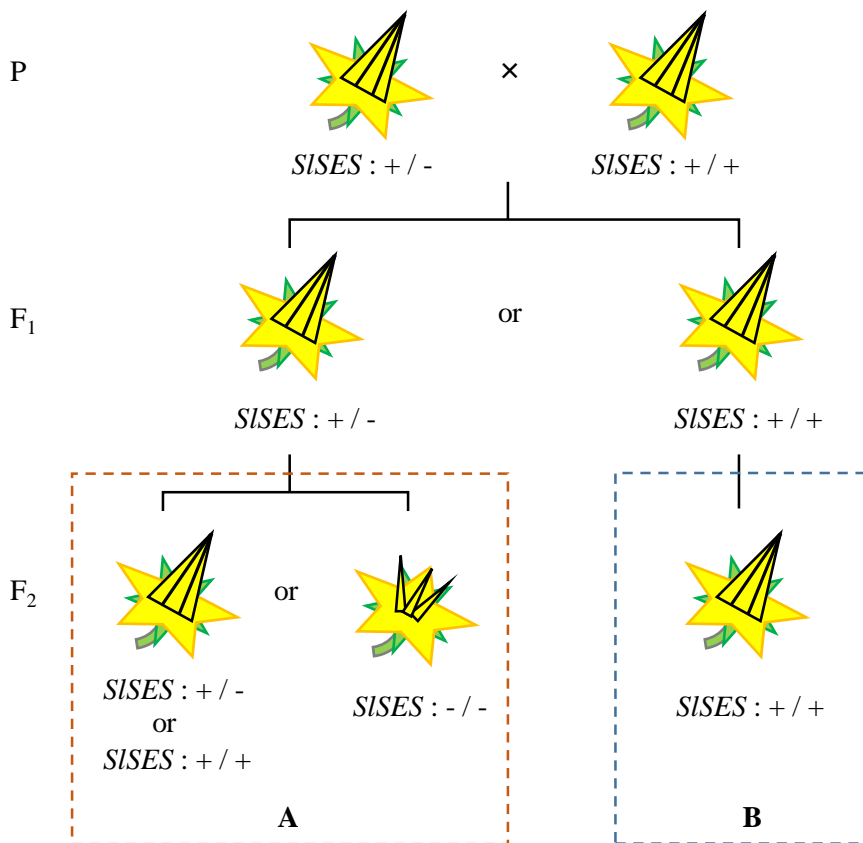
Gene	Solyc ID	Forward Primer Sequence (5'→3')	Reverse Primer Sequence (5'→3')
<i>SISES</i>	<i>Solyc07g063670</i>	AGCCTTTGCTTCACTCATACAGTT	ACTCATCATCGTTGCTTCATTCTC
<i>MS10<sup>35</sup></i>	<i>Solyc02g079810</i>	AAATGACACCAGTGCCACTG	AGTAGATTCCGTGGATGATCTCC
<i>BAM1/2-like</i>	<i>Solyc02g091840</i>	AATTCCCAGGGACACAAAAG	ATAAACCCCCGAGCCTAGAAAG
<i>EMS1/EXS-like</i>	<i>Solyc09g098420</i>	TTTCGCGAACTGTTCAAGCC	ATTGTTGCCGAGTGAGATCCC
<i>TAG1</i>	<i>Solyc02g071730</i>	GCAGAAGAGGGAAGTTGATTTACAC	GTCTAGGGTAATGGTTGTTGGTTTG
<i>SIWUS</i>	<i>Solyc02g083950</i>	AAGAAGAGGCTCATTGCTGCTG	ACCCCATGTGAAGATGGTGATG
<i>SIINO</i>	<i>Solyc05g005240</i>	TACCCCAACATGACTCACAAGC	TTGCTATCTCTTGGGACCATGG
<i>ANT-like</i>	<i>Solyc04g077490</i>	CTGCTGCCTCATTAGTCTTTGC	AAAGACTGGTAGGTGAGCCATG
<i>PIN1-like</i>	<i>Solyc03g118740</i>	CCCACAACCCATAAACCAAAAAC	GCACAACAGCAGTCATAACATGG
<i>BEL1-like</i>	<i>Solyc08g081400</i>	TGATGGAGACTCATCCTTGGAG	CCAGTTTGGCGAGCTAAAATG
<i>SIREV</i>	<i>Solyc11g069470</i>	ACTCGACATGCTGGAGACAAC	AGATACCACCAGGCAAACACG
<i>CNA-like</i>	<i>Solyc03g120910</i>	GTCTAGTGCCGTTTCAGTTTGG	ACCAGCTCTGTTACTCTGCTTC
<i>PHB/PHV-like</i>	<i>Solyc02g024070</i>	GCTCTCGTTCTCTTGGAGGAG	TCTCCTCTCTGTAAACACAAGGC
<i>GOB</i>	<i>Solyc07g062840</i>	TGCATGAATATCGCCTTGATGG	AGTAGCGGCACCATTAGAACC

*SISES*, SEXUAL STERILITY; *MS10<sup>35</sup>*, MALE STERILE 10<sup>35</sup>; *BAM1/2*, BARELY ANY MERISTEM1 and 2;

*EMS1/EXS*, EXCESS MICROSPOROCTES1/EXTRA SPOROGENEOUS CELLS; *TAG1*, TOMATO AGAMOUS1; *SIWUS*, WUSCHEL;

*SIINO*, INNER NO OUTER; *ANT*, AINTEGUMENTA; *PIN1*, PIN-FORMED1; *BEL1*, BELLI; *SIREV*, REVOLUTA; *CNA*, CORONA;

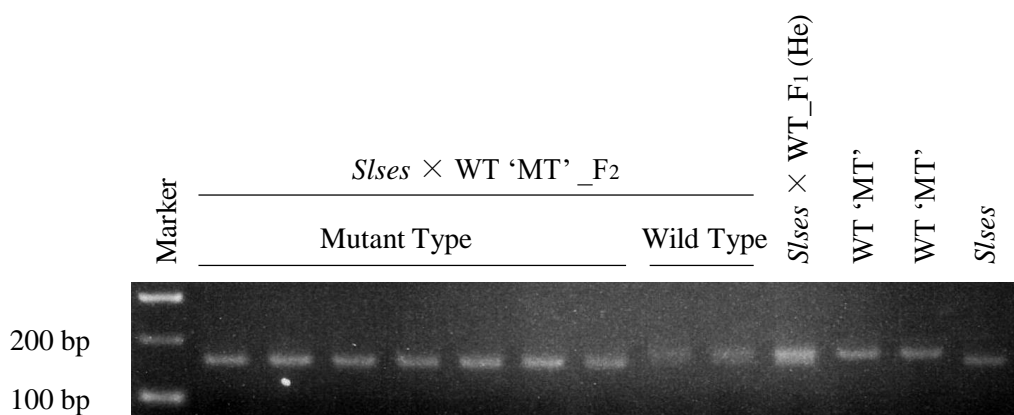
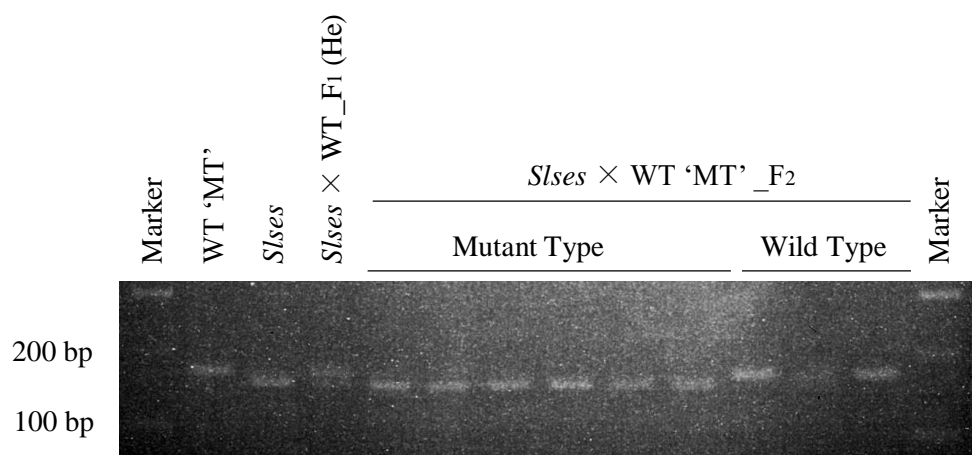
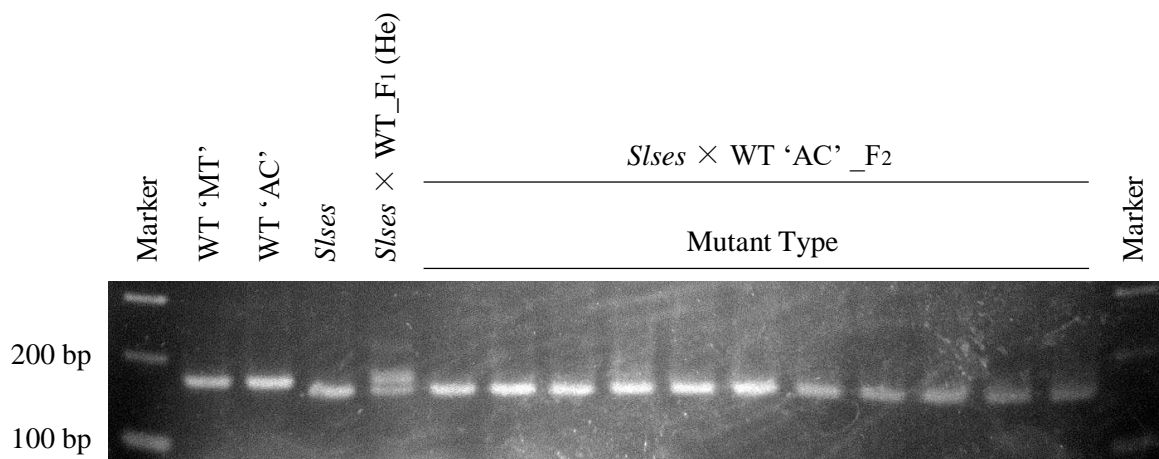
*PHB/PHV*, PHABULOSA/PHAVOLUTA; *GOB*, GOBLET



Supplementary Fig. S1. Schematic diagram of crossing analysis.

The *SISES* homozygous mutant plants showed sexual sterility, and therefore the *SISES* heterozygous mutant plants were used for crossing. All of F<sub>1</sub> plants showed wild type phenotype, but there were two types of genotypes. One type was the *SISES* heterozygous genotype, and F<sub>2</sub> plants from this genotype were segregated to WT phenotype (left image) and mutant phenotype (right image) (A). Another type was WT genotype, and F<sub>2</sub> plants from this genotype showed WT phenotype (B). + indicates wild type *SISES* and - indicates mutated *SISES*.

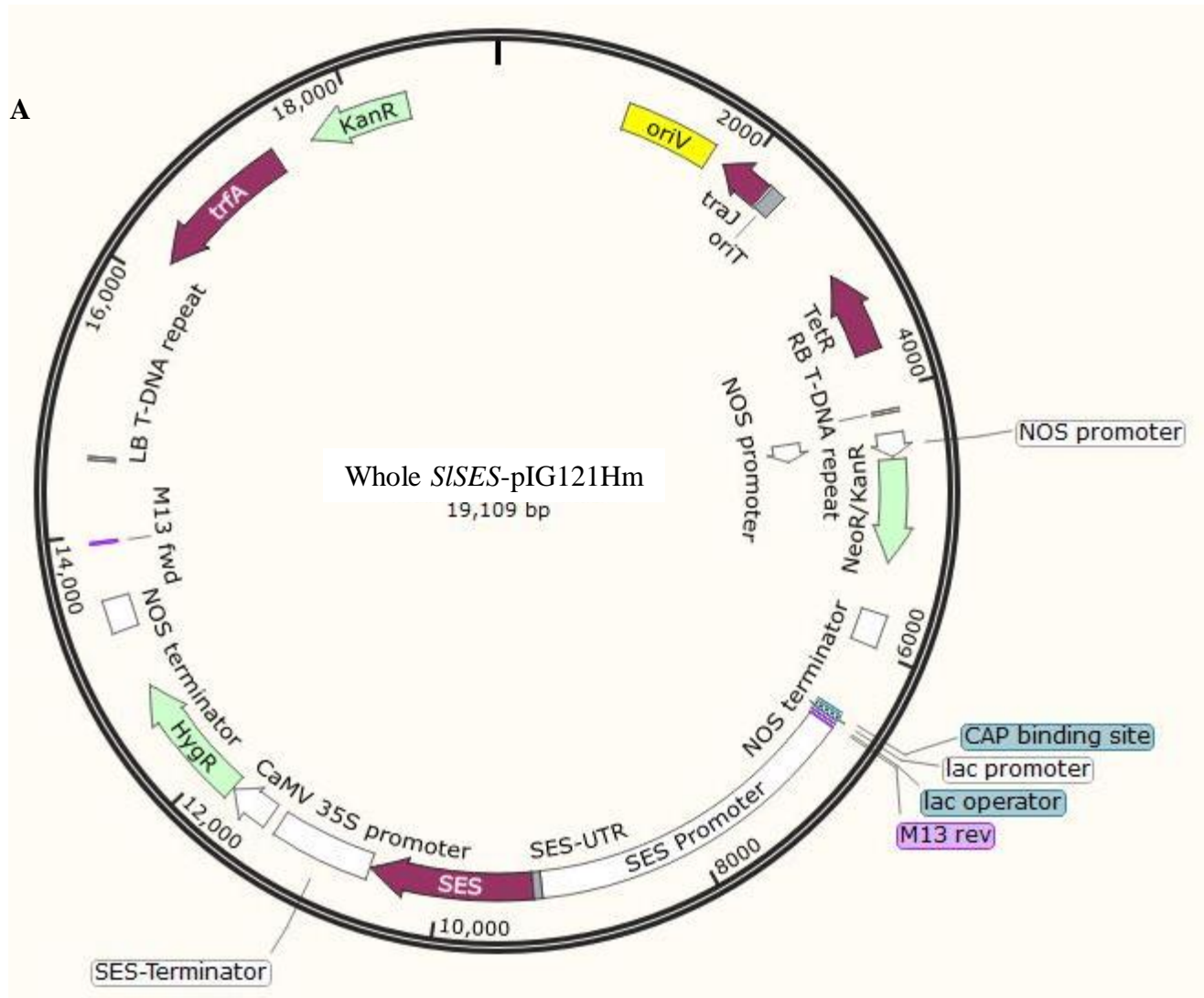




Supplementary Fig. S2. Linkage analysis of the 13-bp deletion on *Solyc07g063670* by *SISES* marker-1.

Micro-Tom WT and Ailsa Craig showed upper band (173-bp), the *slses* mutant showed lower band (160-bp) and the heterozygous showed both bands. All of F<sub>2</sub> plants which showed mutant phenotype had the 13-bp deletion. The F<sub>2</sub> population were constructed from crossed with 'Micro-Tom (MT)' WT or 'Ailsa Craig (AC)'. He, heterozygous.

A

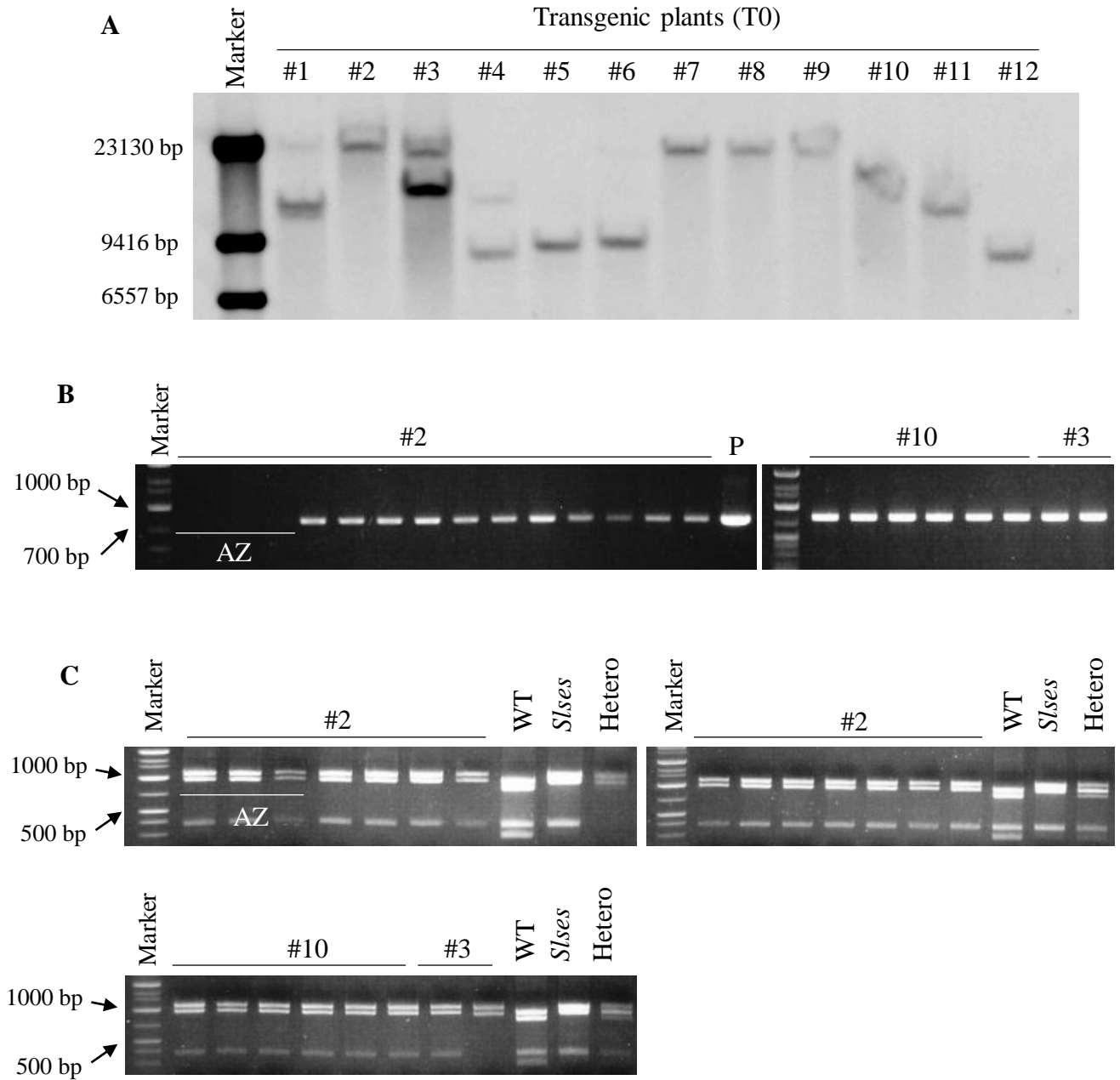


B



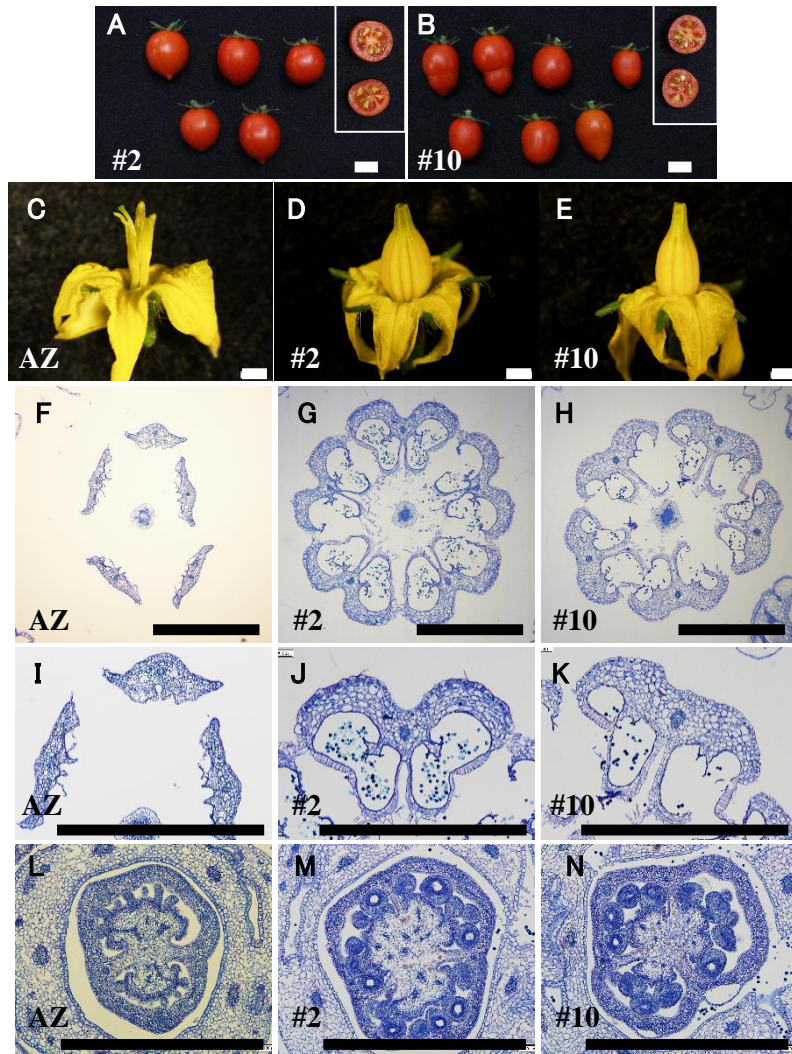
Supplementary Fig. S3. The vector map for the whole *SISES* gene transgenic plant.

(A) Entire vector map constructed from pIG121Hm vector. (B) The transferred region to the plant, from right border (RB) to left border (LB). *NPTII* indicates kanamycin resistance gene. *HPT* indicates hygromycin resistance gene.

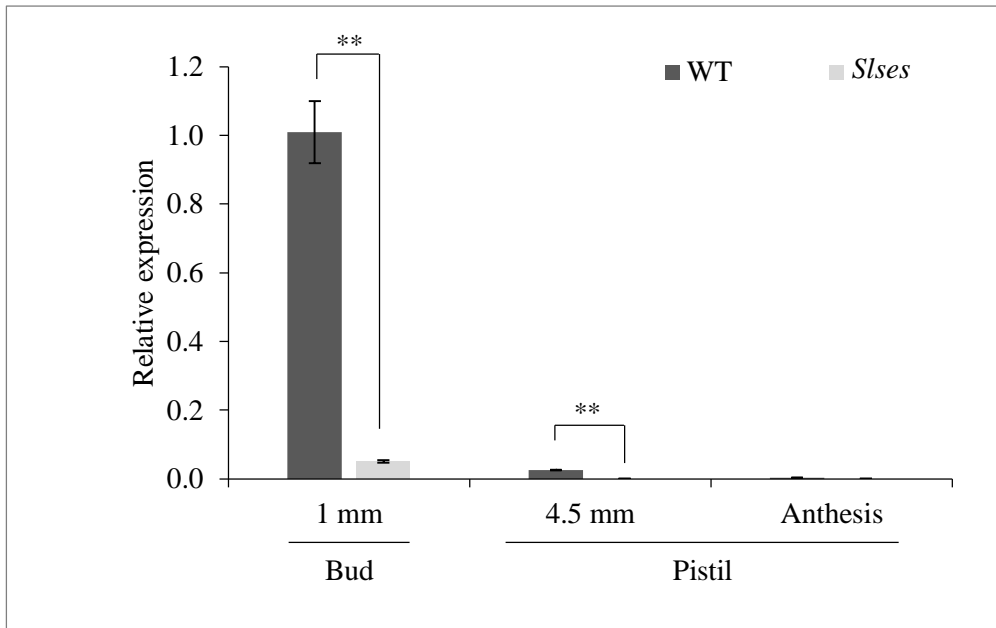


Supplementary Fig. S4. Selection of the plants which not only have transgene but also be the *S/ses* homozygous genotype.

(A) Confirmation of copy numbers of transgene in the T0 plants by southern blotting analysis. The probe recognized *NPTII*. (B) *NPTII* existence confirmation in the T1 plants by *NPTII* marker (See supplementary Table S2). (C) The genotype analysis of the T1 plants by *SISES* marker-2 (See supplementary Table S2). #2, #3, #10 indicate line number and P indicates used vector as a positive control. AZ, azygous; Hetero, heterozygous.



Supplementary Fig. S5. Complementation experiment verified *Solyc07g063670* was responsible for the *SlSes* phenotypes. (A, B) The red ripe fruits of the transgenic *SlSes* mutant in which *SISES* whole gene was induced. Bar = 1 cm. (C-E) The anthesis flower. (F-N) The cross section of the flower at anthesis flower, (F-K) the anther and pollen, (L-N) the ovary and ovules. Bar = 1 mm. (A, D, G, J, M) The transgenic line #2, (B, E, H, K, N) the transgenic line #10, (C, F, I, L) azygous (AZ). AZ plant doesn't have transgenes.



Supplementary Fig. S6. The expression analysis of *SISES* in WT and the *Sises* mutant by qRT-PCR.

The *SISES* expression of the *Sises* mutant at 1 mm bud and 2 stages of pistils from 4.5 mm bud and anthesis stages.  $n = 3$ , error bar = SE, statistical analysis was realized using the Student's *t*-test, \*\* $P < 0.01$ ; \* $P < 0.05$ .